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PUBLIC HEALTH REPORTS.

UNITED STATES.

A REPORT ON LABORATORY WORK IN RELATION TO THE EXAMINATION OF RATS FOR PLAGUE AT SAN FRANCISCO, CAL.

[Prepared by direction of the Surgeon-General by George W. McCoy, Passed Assistant Surgeon,
United States Public Health and Marine-Hospital Service.]

The great importance in a plague campaign of keeping the epizootic in rats under observation is unquestioned. Indeed, sanitarians are at present inclined to attach much more importance from an epidemiological and quarantine point of view to the cases of plague in rats than to cases in man. The reason for this is obvious. Cases in man are apt to be recognized early and such measures may be taken as to render them harmless to the community. With rats, of course, the matter is quite different. Infected animals may be present and scatter their deadly malady far and wide, and the cause of human cases appear to be most mysterious unless careful examination is made of the rats of the community. Thus a systematic examination of rats becomes of prime importance in any scientifically planned plague campaign.

The work of the Indian Plague Commission (Journal of Hygiene, vol. 7, No. 3, July, 1907) has given a new direction to the examination of rats for plague. This commission has by its work put the detection of plague in rats upon a substantial and accurate foundation, as it has shown that plague in rats is attended by certain well-defined lesions easily recognized by the naked eye. It has proved conclusively that the naked-eye diagnosis from the gross lesions is much more likely to be accurate than a diagnosis based upon microscopic findings. These lesions are in brief a bubo, a more or less marked subcutaneous injection, a necrotic or granular condition of the liver, pleural effusion, and a large, firm spleen.

My report is based upon the examination of about 40,000 rats made in March, April, May, and June of the present year, a time when no cases of plague were observed in man. Approximately 98 per cent of the rats examined were classified as belonging to the species, *Mus norvegicus*, the remaining 2 per cent to the species *Mus rattus*. The earlier rat examinations in San Francisco were made the subject of a report by Wherry, Walker, and Howell (Journal American Medical Association, April 11, 1908).

Of the 40,000 rats examined 85 have presented enough of the gross lesions of plague to merit close investigations. Of this number (85) 58 have been shown to be plague infected and 27 have proved negative for plague. All of the infected rats were of the species *Mus norvegicus* except 2, which were of the species *Mus rattus*. Of the 58

infected rats 39 (67 per cent) were so typical macroscopically and microscopically as to warrant a diagnosis of plague without submitting them to the test of inoculation into guinea pigs.

Of the remaining 19 infected rats, 14 presented gross lesions that justified a diagnosis of plague, but the microscopic examination failed to show characteristic organisms, or showed them in such small numbers that for the purpose of confirming the diagnosis their tissues were submitted to the inoculation test. Five (8 per cent) of the infected rats presented gross lesions that made us suspect the existence of plague infection. They were made the subjects of inoculation for the purpose of making a diagnosis. It will be seen that of the 58 infected rats only 5 (8+ per cent) failed to present sufficiently characteristic gross lesions to justify a diagnosis.

Twenty-eight rats that were regarded as more or less suspicious of acute or chronic plague were shown by inoculation experiments to be negative for plague.

The routine procedure was as follows: A rat that presented enough typical gross lesions of plague and showed large numbers of organisms consistent with *B. pestis* in smears from its tissues was recorded as infected, without further investigation. A few of these rats from time to time furnished tissues for the inoculation of guinea pigs for purposes other than those of diagnosis, and in each instance typical cases of plague were produced in the guinea pigs. Rats presenting doubtful lesions, or sufficiently characteristic lesions but without organisms consistent with *B. pestis*, were recorded as suspicious until the results of guinea-pig inoculation made a positive diagnosis possible. On only a few occasions were cultures made directly from an infected rat. I believe sufficient reason existed for this because no artificial medium is so good for the growth of *B. pestis* in the presence of contaminating organisms as is the body of a living guinea pig, and in dealing with rats, the tissues of which were usually invaded with other organisms by the time they reached us, the securing of a pure culture of *B. pestis* by culture methods alone would have taken more time than we had at our disposal. Cultures were made from guinea pigs, and of course no difficulty was experienced in isolating the bacillus and studying it.

A word should be said here about the search for the bacillus in the tissues of infected rats. We have not considered it wise to spend much time in the search for the organism, and it was only when the typical organisms were present in considerable numbers or in almost pure culture that we permitted the microscopic finding to have any material weight in arriving at a diagnosis.

Rats, in general, that are somewhat decomposed will very frequently show a multitude of bacterial forms in the tissues, many of which are not to be distinguished morphologically from *B. pestis*. It is, of course, well known that great care should be taken in drawing conclusions from the morphology of organisms in general, and P. A. Surg. M. J. White (Medical Record, January 28, 1905) has emphasized this, especially in regard to plague in rats. The fallacy of a diagnosis based on morphology has been made very clear to us in the following manner: It is extremely desirable that a method be found for the diagnosis of rat plague in doubtful cases without awaiting the result of guinea-pig inoculation, and it seemed to me that the well-known ability of *B. pestis* to multiply vigorously at a lower

temperature than do many other bacterial species might be taken advantage of in making a diagnosis in such cases without awaiting the death of the inoculated animals. Occasionally when a rat with suspicious gross lesions was encountered in which plague-like organisms were absent or were found in such small numbers as to leave doubt in my mind as to their nature, we put the liver and spleen aside in a dark place at a temperature of from about 18° C. to about 22° C. Smears from the tissues were examined in 24 hours and again in 48 hours. In every case guinea pigs were inoculated for the purpose of controlling the method. In the first four cases apparently brilliant results were obtained. The tissues incubated at a low temperature showed practically a pure culture of *B. pestis*, and the guinea-pig controls died of plague. In the fifth case, what appeared to be a perfectly typical growth of *B. pestis* in the tissues in pure culture was obtained, but the guinea pigs failed to develop plague. In the sixth case the reverse happened; that is, the tissues failed to give a growth which we were willing to call *B. pestis*, yet the guinea pigs died of plague.

The method used in inoculating the tissues of suspected rats is as follows: The belly of one guinea pig is shaved over an area about 3 cm. square and over a part of this area, perhaps 1 cm. square, the shaving is done in such a manner as to abrade the epithelium, leaving a raw surface. In accordance with the injunction of the Indian Plague Commission, no soap or water is used; but I may say, in passing, that before I became acquainted with the views of the Indian Commission I frequently used soap and water in preparing guinea pigs for inoculation by this method, and have never seen any failures attributable to that procedure. On this abraded area, a piece of tissue (spleen, bubo, or liver) from the suspected rat is rubbed, using but little pressure. A second guinea pig is inoculated by a procedure used at the Hygienic Laboratory of this Service. In this procedure an incision about 0.7 cm. in length is made in the skin of the belly wall, a sterile dressing forceps is thrust down between the skin and the muscles for about 4 cm., and well into the pocket thus formed a bit of suspected tissue is placed. When guinea pigs are inoculated from an infected rat by the first procedure given above death usually occurs in four or five days (average, 4.8 days); by the second procedure, death usually follows on the third or fourth day (average, 3.6 days). I strongly advise against inoculation with a syringe, as when this is practiced a considerable number of guinea pigs will die acutely of infections other than plague.

THE NAKED-EYE APPEARANCE OF PLAGUE IN RATS.

The bubo.—Of the 58 infected rats, 32 (55 per cent) presented one or more well-defined buboes; 11 (19 per cent) presented no definite bubo, but a general enlargement of the lymph glands; 15 (26 per cent) presented no gross lesions of the lymph glands. Of the buboes, 26 were more or less softened or caseous upon section, while 4 were distinctly hemorrhagic. All were quite firm when examined before section, and the softened contents could be shelled out, leaving a well-defined capsule. One gland was white, succulent-looking, and not injected.

Buboes were single in 29 instances. Of these, 21 were situated in the groin, 6 in the axilla, and 2 in the pelvis. Two rats were met with

in which multiple buboes occurred. In one the right axillary and left inguinal glands were involved, and another had bilateral inguinal involvement. It is a remarkable and noteworthy fact that the cervical bubo which occurred in 72 per cent of natural plague rats examined by the Indian Commission was not found once in this series. A typical submaxillary bubo is recorded once in the 88 cases reported by Wherry, Walker, and Howell, and 33 times the submaxillary glands were reported as enlarged and congested. I am disposed to believe that some important and as yet not understood fact lies at the bottom of this difference between the experience here and that in India. Where note was made of the presence of bacilli in the buboes, they were found, sixteen times; absent, four times. In five of the sixteen cases in which bacilli were found, the "coccoid" form predominated.

Enlarged lymphatic glands are exceedingly common in rats, and glands that are merely enlarged, without surrounding infiltration or injection, are, in our experience, of no significance whatever.

Subcutaneous injection.—This sign was noted as present in greater or less degree forty-nine times (84+ per cent), in forty-five of which it was general in distribution. Twice it was confined to one side of the body, and in each of these cases it was found on the same side with the bubo. In one case no injection was noted except in the neighborhood of the glands. The injection was noted as slight eleven times; moderate, fifteen times; marked, sixteen times; and intense, seven times. In only three cases was it recorded as absent. In one case there was a considerable area of œdema in the axilla and the adjacent chest wall. The injection is the sign we have seen most frequently. When typical it is very highly significant of plague. The color is a rather dusky red. A bright pink injection is quite common in rats here other than those infected with plague.

The Indian Commission found subcutaneous injection in 69 per cent of their cases. It was recorded as present in 59 per cent of the cases reported by Wherry, Walker, and Howell.

Liver signs.—The liver was recorded as showing lesions in fifty cases (85+ per cent). Of these cases, in twenty-six the liver was yellowish brown in color and presented very distinct yellowish granules varying in number from very few to an enormous number and in size from a mere point to that of a mustard seed. In fifteen instances small whitish yellow areas rather indistinct in outline were seen. In these cases the liver showed no other departure from the normal. In two of the latter cases the spots were confined to the margin of the organ.

In 4,000 infected rats the Indian Commission found lesions of the liver in 58 per cent of the cases. Wherry, Walker, and Howell recorded them in 15 per cent of their cases.

Pleural effusion.—This sign was present thirty-four times (58+ per cent); absent, fifteen times; and in six cases the thorax was so injured by the trap that we were unable to say as to the existence of pleural effusion. The effusion was clear, serous in character, twenty-three times; blood-tinged or bloody, eleven times. Some of the latter may have been due to an injury to blood vessels when the rat was dissected. The effusion was small or moderate seventeen times; large, fifteen times.

The Indian Commission found pleural effusion in 72 per cent of cases. Wherry, Walker, and Howell found it in 71 per cent.

The spleen.—This organ was enlarged forty-two times; not enlarged, thirteen times. In consistency it was firm thirty-seven times; soft, fifteen times. The color was deep-red forty times; slate-colored, nine times; and mottled, twice. The splenic signs, in my opinion, are less useful than any of the others. This is also the view expressed by the Indian Commission. After a considerable experience in the examination of rats, I have no very clear idea as to what the normal size of the spleen should be. It varies in rats apparently healthy and of about the same size from 3 cm. in length up to 6 cm., and its other dimensions are equally variable.

In this series of 58 infected rats no one sign was found in every case in the series. In twenty-three instances (40 per cent) all the gross lesions of plague in rats were present. A typical bubo is the only lesion existing alone on which I would be willing to hazard a diagnosis of rat plague. Without a typical bubo, the other signs should be well marked to justify a diagnosis of plague.

The cases that proved negative.—Fifteen rats were found presenting microscopic lesions more or less suspicious of acute plague. Thirteen of these rats presented granular and necrotic foci in the liver, which on two or three occasions appeared to be identical with those found in plague. In two cases an intense congestion was the only markedly suspicious sign. Pleural effusion was met with four times. It is noteworthy that in not one of these cases was a typical bubo found, and I may say in passing that in no case in which a typical bubo has been found has plague not been confirmed when inoculations have been made.

Only one of these fifteen rats was recorded, after gross examination, as probably plague infected; in this case a marked subcutaneous injection, a typical plague liver, a large, firm, deep red spleen, the axillary and inguinal glands on one side deeply injected, and a large, clear, serous, pleural effusion, made a very suspicious combination. In addition, a few bipolar organisms were found in smears. Two guinea pigs were inoculated from the spleen of this rat by the routine method. They were both killed on the tenth day and in each case were found normal beyond the presence of a few whitish-yellow granules in the spleen. Smears showed a very few solidly staining organisms in the spleen resembling *B. typhosus*. Cultures made were negative for *B. pestis*, but gave a growth of an organism that I have not completely identified. This same organism, or a similar one, has been encountered in another rat presenting in the liver lesions suspicious of plague. It does not appear to be at all closely related to *B. pestis*.

Of the negative cases, twelve were rats that presented nothing suspicious of acute plague, but presented suppurating or caseous foci that were regarded as possibly due to chronic plague. Eleven of these foci were found to be suppurating or caseous lymphatic glands. They presented nothing in smears that could be interpreted as *B. pestis*. They were in each case tested on guinea pigs, but always with negative results. The remaining case was one that we were disposed to regard as very suspicious of chronic plague, as described by the Indian plague commission. The rat presented no lesion, except a caseous mass (abscess) adherent to the lower edge of the spleen. The lesion was about 1 cm. in diameter. The abscess cavity was filled with a semisolid yellowish mass. No organisms were found in smears, and

the inoculation of two guinea pigs resulted negatively. We have encountered in our work here no example of chronic or latent plague.

Cultural characteristics.—The organisms I have isolated here all gave the reactions for *B. pestis* on artificial media—that is, they did not liquefy gelatine, or ferment glucose, lactose, or muscle sugars. Milk was unchanged. The growth on agar was the characteristic sticky, translucent film usually given by plague. Only on broth and salt agar did plague give growths that could be considered at all characteristic.

We have not prepared brotl. especially for the development of stalactites—that is, by the use of an overlying fat—but have used ordinary broth in which the growth is sufficiently characteristic. Under these conditions a fine, more or less granular, precipitate was formed, which adhered to the sides and bottom of the tube. The medium never showed uniform clouding when the culture was grown under conditions that precluded serious vibration. On the surface was found a delicate, patchy film, or often only a few islands of growth; and upon slight agitation there fell down from the surface film delicate, globular masses of the growth, often suspended by a fine filament from the surface. I observed one or two old cultures that gave what appeared to be a rather uniform turbidity; however, upon close inspection, it was found that the culture was full of exceedingly fine flakes, rather than that a uniform turbidity existed. In the old cultures a rather heavy scum was observed at times, which I was inclined at first to regard as evidence of contamination; but further investigation in every case showed these tubes to contain pure cultures.

Involution forms on 3 per cent salt agar.—The production of involution forms is, of course, universally regarded as of the greatest value in establishing the identity of a given organism such as *B. pestis*. My experience has amply confirmed this. In some thirty-odd cultures of *B. pestis* isolated here from plague-infected rats, usually after passage through guinea pigs, we never failed to get characteristic involution forms. These forms were generally to be found on 3 per cent salt agar after twenty-four hours, and always typically after forty-eight hours. The gigantic globose and trypanosome-shaped forms have, as is well known, no resemblance at all to the ordinary forms of *B. pestis*, and I more than once questioned whether we were dealing with plague at all when such extreme involution forms were present. However, I often recovered pure cultures of the ordinary type from these extreme involution forms by transplanting to ordinary media.

A few other organisms give forms that resemble the involution forms of *B. pestis*; notably *B. diphtheriæ* and *B. mallei*. The diphtheria organism does not produce forms within forty-eight hours that should lead to any confusion, but with that of glanders the case is different, as it will in forty-eight hours, or in even less time, give the long whip-like forms. Of course, the other points of difference between the two organisms would preclude any possibility of confusion.

There are several points to be observed in the use of salt agar if we are to obtain trustworthy results. In the first place, the salt used should be chemically pure sodium chloride. When I first began to work with plague cultures at San Francisco, I found that upon salt agar (3 per cent) I could get no growth at all, and, in fact, the organisms grew very poorly upon all my media. Different lots of salt agar were made, beef, beef extracts, tap water, distilled water, and differ-

ent reactions to phenolthalein being used, but still upon the 3 per cent salt medium I could get no growth of plague. My technique was carefully reviewed and the antecedents of my materials were considered. Finally it was learned that the salt was ordinary table salt from a grocery, which had inadvertently been put into a container marked "chemically pure sodium chloride." When a new lot of really pure salt was obtained no further difficulty was experienced.

A sample of this salt which so strongly inhibited the growth of *B. pestis* has been submitted to the Hygienic Laboratory of the Public Health and Marine-Hospital Service for chemical analysis, and I propose to carry out a series of experiments to determine what impurity or impurities led to the failure of plague upon this media.

The second point learned from our experience here is, that it will very frequently happen that upon salt agar (3 per cent) only a very feeble growth or even no growth, is obtained when the medium is inoculated directly from an animal. When the inoculation is made upon broth or ordinary agar and a generation of the organism is so obtained, it may be transplanted to the salt agar and a vigorous and satisfactory growth be assured.

A third point less definite and well established than the two previous ones is the variation in the type of involution-forms, dependent upon the length of time during which the culture has been carried upon artificial media. It has been my experience that cultures of plague recently isolated from an infected rat show marked involution forms earlier than a culture long carried on artificial media. Indeed, some old cultures, even after forty-eight hours on salt agar, show a large number of the organisms to be but moderately different from the forms on ordinary agar. Another point of difference between old and recent cultures is that in the former the large "whip" forms are apt to predominate, while in the recently isolated cultures globose and spindle-shaped forms usually predominate.

While there has been no question raised in any responsible quarter as to the existence of plague among the rats in San Francisco, it may not be amiss to make mention of the steps taken to absolutely prove the existence of the disease so far as methods at our disposal made it possible. In 2 cases, from rats which from gross appearances were considered plague-infected and which showed in smears from their tissues, organisms consistent with *B. pestis*, guinea pigs were inoculated by the cutaneous ("vaccination") method. The guinea pigs died on the 4th and 5th days, respectively. The animals presented the typical lesions of plague on post-mortem examination. In each case an organism was isolated from the heart's blood which gave all the cultural reactions of *B. pestis*, special attention being paid to the growth in broth, and to the production of involution-forms on salt agar. Cultures of these organisms (third generation) were then used to inoculate guinea pigs, again by the cutaneous method. One loopful of a 72-hour agar culture was used on each guinea pig. Two guinea pigs were inoculated with each culture, one having been given, intraperitoneally, just before the inoculation, 2 cc. of antiplague serum from the Pasteur Institute in Paris. In the case of culture A, the animal that had not received an inoculation of serum, died on the 7th day, while the animal that received the serum lived 12 days. In the case of culture B, the animal that received the serum did not sicken at all. It was killed on the 30th day after inoculation and at necropsy presented only

a small caseous gland in the left groin in which no *B. pestis* could be found. The control died on the 8th day. The 3 animals that died showed typical lesions of plague. In each case a pure culture of an organism giving all the reactions of *B. pestis* was obtained from the heart's blood of the dead animal. It is perhaps of not much significance that in the case of culture A, the guinea pig protected by serum lived 5 days longer than the control. The protected animal inoculated with culture B, was, however, the only example I have encountered of a guinea pig that survived inoculation with any of the cultures of *B. pestis* isolated at San Francisco. These two cultures, A and B, are the only ones I have isolated here that were tried against antiplague serum. The serum used has been in stock for a number of months and I am inclined to believe that its protective value is not especially high.

I wish to express my indebtedness to Acting Assistant Surgeon W. B. Wherry for valuable suggestions, and for kindly placing at my disposal plague literature otherwise not available.

[Reports to the Surgeon-General, Public Health and Marine-Hospital Service.]

Reports from San Francisco, Cal.—Plague prevention work at San Francisco, Oakland, and Emeryville, Cal.

Passed Assistant Surgeon Blue reports:

SAN FRANCISCO, CAL.

Week ended July 3, 1908.

Date of last case.....	Sickened, January 30, 1908
Sick inspected.....	9
Dead inspected.....	83
Premises inspected.....	15, 726
Houses disinfected.....	103
Houses destroyed.....	17
Nuisances abated.....	1, 468
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Rats found dead.....	432
Rats trapped.....	3, 251
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Total rats taken.....	3, 683
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Rats identified:	
<i>Mus norvegicus</i>	2, 621
<i>Mus rattus</i>	40
<i>Mus musculus</i>	379
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Total.....	3, 040
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Rats examined bacteriologically.....	2, 413
Rats infected with <i>B. pestis</i>	1
Poisons placed.....	85, 882

Week ended July 11.

Sick inspected.....	20
Dead inspected.....	139
Premises inspected.....	14, 762
Houses disinfected.....	132
Houses destroyed.....	6
Nuisances abated.....	2, 003
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Rats found dead.....	437
Rats trapped.....	3, 923
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Total rats taken.....	4, 360
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